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A promising therapeutic approach to spinal cord repair

Every year 30 000 neuroscientists meet in the USA. Few cities have the facilities to accommodate them all. Their proceedings fill the equivalent of more than three London telephone directories. A patient with spinal cord injury may wonder if he is living on the same planet. How come he is still confined to a wheelchair? Is it simply the magnitude of the problem? Or is there some inbuilt pessimism that is retarding advance?

Many tissues of the body are capable of self-repair. Skin, bone, gut, and others can recover from injury. But despite all efforts of the patient and the medical profession, the tissues of the brain and spinal cord—which together constitute the central nervous system—show major and irreversible loss of function if they are damaged.

In approaching this matter we encounter two striking and prevalent ideas. The first is that, although the embryonic central nervous system is able to form new connections, it loses the ability at some time after birth.[1] The second is that the adult central nervous system is full of inhibitory molecules which prevent regeneration of damaged nerve fibres.[2] Together these truly represent a pessimistic view. But let us explore them.

The first idea—that the mature nervous system cannot form new connections—flies in the face of what we understand to be the purpose of the spinal cord. We think of the brain at birth as a sort of *tabula rasa*, a white sheet of paper, ready to have imprinted upon it all the skills that will serve us in later life. And this learning process goes on throughout life. It seems perverse to imagine that all this adaptation takes place in an organ that is itself static, incapable of change, if not already senescent. Indeed, for those wishing to take another view, there have always been more optimistic indications. When nerve fibres are cut, they sprout vigorously, even though unable to find their way back to their original destinations.[3] When nerve cells lose connections, new connections are formed by adjacent nerve fibres, restoring exactly the original number of connections that existed before the injury.[4] Functions which excite one part of the brain can, as a result of damage, come to reside in different areas.[5,6] From a functional point of view, the adult nervous system is in a state of continual change. The goal of research is to work with and build further upon this intrinsic plasticity—to devise new therapeutic interventions that will lead to the recovery of functions which currently do not return after injury.

The second idea—that the adult central nervous system is full of inhibitory molecules which prevent regeneration of damaged nerve fibres—originated from observations made on nervous tissue grown in culture.[7] Growing axons were seen to be repelled by certain cells (such as oligodendrocytes) or molecules (such as myelin). But if the central nervous system is full of inhibitory molecules, how could learning and plasticity occur? Is it possible that what is manifested as inhibition in tissue culture actually represents a response to molecules whose function in the intact nervous system is to guide axons, not to prevent their growth? This guidance theory has important implications for an alternative strategy in central nervous system repair—that of antagonizing the supposedly inhibitory molecules. If they are essential for guiding nerve fibre growth, then knocking them out will not help repair, but prevent it.

Let us make the seemingly heretical assumption that the environment of the brain and spinal cord is propitious for the growth of nerve fibres. How then can we explain the fact that severed nerve fibre tracts are not regenerated? Why are damaged nerve fibres not capable of restoring connections that in earlier life were established during normal development? To understand this, we need to consider what happens during development. The nervous system begins life as a simple one-cell-thick plate of cells which becomes folded into a hollow tube. By subsequent divisions of cells, migrations and differentiation, the immensely complex structure of the adult brain and spinal cord is achieved. Looking at the pathway taken by nerve fibres during development, we see that the different tracts of nerve fibres do not grow all at the same time but sequentially. And at the time they are growing, nerve fibres follow relatively straightforward pathways. Largely, in fact, they simply travel under the outer surface of the neural tube.[8] The fibre finds its correct pathway by means of the growth cone, a sensitive structure rather like a groping hand, located at its leading edge. The ever-moving fingers of the growth cone explore the environment, detecting and responding to intermediate or 'guidepost' cues on the way to the final target. But the preponderant cues are negative ones—in other words, the natural exuberance of the growing fibre is constrained to its correct pathway by being

repelled from making incorrect decisions. And when it reaches the correct target, the signal it receives is a negative one—stop growth. In a system whose default response is to grow, order is achieved by a hierarchy of negative signals. They do not antagonize growth: on the contrary, they are essential to impose order and guidance upon it.

Now, when a nerve fibre is damaged it becomes released from the natural restraints, and resumes its default pathway which is growth; hence the neuromatous mass of sprouts seen at injury sites. But in the adult the growing fibres are confronted with an environment totally different from that in the embryo. Apart from the much larger distances to the target, the whole structure of the tissue has been changed during development by the continuous formation of new cells, their migrations, the formation and continuous overlaying of new fibre systems, and the folding and twisting of the brain itself. Even if all the molecular cues required for guidance are present in the adult, and even though, as we can see, the cut nerve fibres make every attempt to grow, the original pathways are simply not present any longer. And it is difficult to see how simply adding or subtracting one or other molecule will replace them. The nervous system is a highly ordered structure. Regeneration will only occur if the defects in that order are somehow repaired. How may that be done?

We will begin by considering what happens when nerve fibres are damaged. The huge majority of the cells in the brain and spinal cord are not nerve cells but glial cells. Glial cells come in at least three types—astrocytes, oligodendrocytes, and microglia—and the different cells are arranged in networks and channels of almost crystalline regularity.[9] We can think of them as a highly structured and living society. During development the glial cells are the major carriers of the guidepost signals which allow nerve fibres to reach their correct destinations.[10] They form the aligned pathways along which nerve fibres grow.[11] When damage occurs in the adult, the three types of glial cells behave quite differently: astrocytes swell and form reduplicated layers, oligodendrocytes die, and microglia migrate into the area of damage.[12] Moreover, the blood–brain barrier is breached and there is an influx of blood-borne cells. As a result, the site of injury becomes transformed into a complex abnormal tissue, usually called a scar, quite unlike anything that growing nerve fibres encounter in their normal life. Not surprisingly, they are unable to find the channels and guideposts that they are programmed to detect.

So what can we do to make the glial scar penetrable? How can we bridge the gap? And if we did so, would nerve fibres be able to cross the newly constructed bridge and continue through their original pathways and re-establish function? At least in some experimental circumstances this has been achieved.[13] One of the main means used to bridge areas of damage is the transplantation of cells. The idea is to take cells from areas where nerve fibres can grow, and transplant them into areas where they cannot. Three types of transplanted cells have been examined: (*a*) cells from the embryonic nervous system (i.e. taken at a stage in development when nerve fibres are normally growing);[14] (*b*) adult Schwann cells from peripheral nerves (where cut nerve fibres are able to grow);[15] and (*c*) ensheathing cells from adult olfactory nerves (which are in a state of continuous growth and replacement throughout adult life).[16] For the rest of this article, I will describe some experiments from my own team, based on transplants of these olfactory ensheathing cells, currently one of the most promising routes for repair. These cells have the particular advantage that they may be obtained from adult patients, who can thus build up a bank of their own cells for autografting into areas of damage in the spinal cord.

What is so special about cells from the olfactory system? For many years it was thought that we are born with a full complement of nerve cells, and that the only change we can expect during life is to lose them. But the advent of labels to detect cells undergoing division showed that new neurons are continually added to the olfactory system of the adult brain[18]—an initially derided finding that took 20 years to become accepted. The same methodology also revealed that the neurosensory cells of the olfactory mucosa have a life of about thirty days, and are continually replaced by the progeny of mucosal stem cells generated throughout adult life.[19] The continuous death and replacement of olfactory neurons means that the olfactory nerves are likewise in a state of continuous replacement. And this raises the question of how these newly formed nerves are able to enter the brain. In 1985 I described a unique arrangement of specialized olfactory ensheathing glial cells that accompany the olfactory nerve fibres all the way to their entry into the brain.[20] The subsequent development of a tissue culture method[21] for obtaining olfactory ensheathing cells from adult olfactory tissue samples led to a mixture of two main types of cells, some Schwann-like, others fibroblast-like, which could be transplanted into experimental lesions of the spinal cord.

In our own studies we transplanted this mixture of cultured olfactory ensheathing cells into complete unilateral lesions of the upper cervical corticospinal tract in adult rats.[22] We found that the grafted cells encourage the growth of the cut nerve fibres, and suppress the excessive neuromatous branching found in untreated lesions. The grafted cells take up an elongated shape and form a tightly aligned bridge between the ends of the cut fibre tract. The regenerating nerve fibres enter the graft and follow this new aligned bridge pathway. Within the bridge the nerve fibres are intimately ensheathed by the Schwann-like cells, and enclosed in an outer, perineurial-like, sheath of fibroblasts.

But, most important, once they reach the end of the graft they re-enter the host spinal cord and continue along the distal part of the corticospinal tract to form terminal arborization in their normal target areas. During their course through the transplant the fibres are myelinated by peripheral myelin formed by the Schwann-like cells, and when they re-enter the spinal cord they are myelinated by host oligodendrocytes. The effect is to put a patch over the lesion, restoring the integrity of the original pathway. This regeneration can be achieved by transplanting at some time after the original injury, and leads to the functional recovery of a learned task. In a second series of investigations[23] we studied the effects of complete hemisection of one half of the spinal cord at the upper cervical level. This results in a deficit in the use of the ipsilateral forepaw in a climbing test and the disappearance of the supraspinal respiratory rhythm in the ipsilateral phrenic nerve and hemidiaphragm. Both these functions can be restored after transplantation of cultured olfactory ensheathing cells.

The rat results are encouraging, but at some point we have to decide whether the system really is predictive of human repair. The steps to be taken include finding how to obtain sufficient numbers of reparative cells from human nasal mucosal biopsies, and devising a method of surgical implantation that will not increase the damage but will enable the limited numbers of cells we can hope to obtain to form a continuous anatomical bridge. It will also be necessary to define the exact conditions which will be the basis of a first, limited, trial. The patient's history must be such that at the time of intervention no further spontaneous recovery can be expected, and the proposed operation must be sufficiently atraumatic that no further loss of nervous functions will be caused by the intervention. We are currently in discussion with the National Hospital for Neurology and Neurosurgery at Queen Square, and hope that these desiderata may be fulfilled in the next period. The proof of all this speculation will only come when a patient has recovered functions that cannot be restored by any existing techniques.

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